

Lysosomal Storage Disorders

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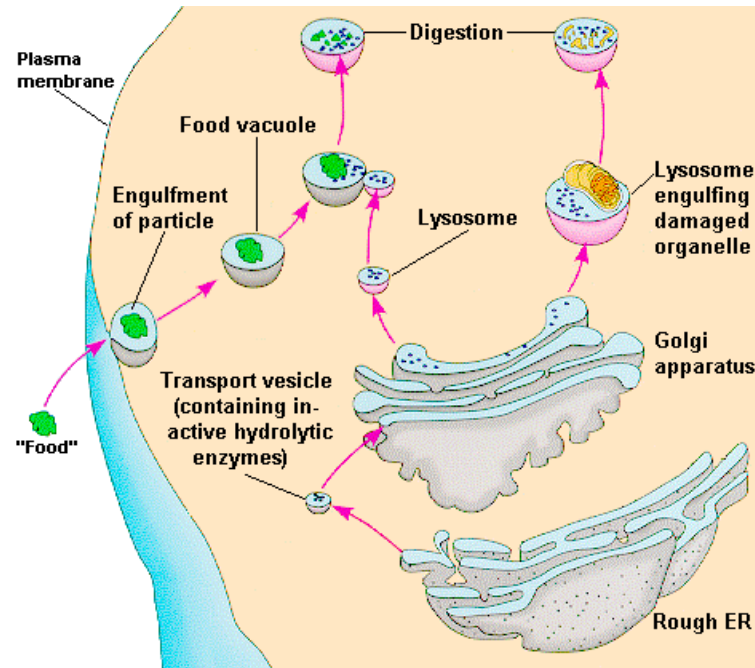
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Lysosomes



Lysosomes are cellular membrane-bound organelles that are present in all nucleated cells. These are vesicles with acidic contents (pH 4.5–5.5) maintained by an ATP driven proton [H⁺] pump on the lysosomal membrane. This is the optimal condition for numerous hydrolytic enzymes to process ingested materials and aged or damaged organelles into substances that the cell can re-utilise. Therefore lysosomes are sometimes known as the ‘cell recycling centre’.

Lysosomal Enzymes



Lysosomal enzymes are formed in the rough ER with high mannose oligosaccharide residue(s) attached. They are transported to the Golgi apparatus where they undergo further modifications. This involves attachment of a terminal mannose-6-phosphate group which binds to mannose-6-P receptor and enables transfer of the enzyme to primary lysosome. In the acidic environment the enzyme is dissociated from the receptor and becomes active. The mannose-6-P group is therefore an important recognition marker for the enzyme to be packaged in the lysosome.

Lysosomal Storage Disorders

Up to now there are some 40 different lysosomal storage disorders (LSD) known in humans. The majority of LSD are caused by a deficiency of a single lysosomal enzyme. A few LSD are caused by defective transporter or protein activator activity, indirectly contributing to the enzyme deficiency. All defects invariably result in 'storage' of partially degraded substances within the lysosomes, consequently disturbing cell homeostasis and hence lead to early cell death.

LSD are classified by the type of storage material involved

<i>Groups of LSD</i>	<i>Types of material accumulated</i>	<i>Examples</i>
Mucopolysaccharidoses	Glycosaminoglycans	Hurler, Scheie, Hunter, Sanfillipo, Morquio, Maroteaux-Lamy and Sly Syndromes
Oligosaccharidoses	Oligosaccharides	Fucosidosis, Mannosidosis, Sialidosis, Schindler's Disease, I-cell disease, Aspartylglucosaminuria
Sphingolipidoses	Membrane glycosphingolipids	GM1 gangliosidosis, GM2 gangliosidosis (Tay Sachs and Sandhoffs Disease), Fabrys, Gaucher's Niemann Pick A and B Diseases. Metachromatic and Krabbe's Leucodystrophies.
Others		Pompe's Disease, Niemann Pick C, Mucopolipidosis IV Wolmans Disease, Ceroid Lipofuscinosis (Battens Disease), Cystinosis, Salla disease

LSD: Disease Nature

Most LSD are autosomal recessive disorders. The typical disease course of LSD is progressive and unremitting. Patients are usually well at birth and manifest in the first 3-6 months. However there is enormous clinical heterogeneity in this group of disorder. At the most severe end of the spectrum there are neonatal Gaucher and I-cell diseases which present in the newborn period or die in utero; at the other end there are the heterozygous female Fabry patients who may remain asymptomatic throughout life. Moreover, disease severity appears to be associated with residual enzyme activity. In a few LSD some genotype-phenotype correlations are established, this may be useful to design treatment strategy e.g. MPS I.

The underlying mechanisms that give rise to the pathological effects in LSD is still unclear but the distribution of the storage material will determine which organs are affected. Cells with low turnover rates (e.g. neurons) or those rich in lysosomes (Mononuclear phagocyte system) are most vulnerable to damage by the accumulation of storage material. Most LSD present with neurological symptoms such as movement disorders, mental retardation, behavioural changes, seizures, dementia, deafness or blindness. Developmental and milestone regression frequently occur. Hepatosplenomegaly, pulmonary and cardiac problems and bone abnormalities may also be the main presenting symptoms. Most LSD will slowly progress to a life-threatening, multi-organ disease.

LSD: Laboratory Investigations

The resultant intracellular morphological changes are demonstrable by histochemical techniques on blood films or on biopsies of affected tissue, although the latter is required to confirm a diagnosis in only a few LSD.

The storage material leaks into tissues and is excreted in large quantities in the urine. A simple urine screening test for glycosaminoglycans (mucopolysaccharides) is offered by some laboratories although it has limited sensitivity and specificity. Most laboratories perform the more reliable technique of urine glycosaminoglycan cellulose acetate electrophoresis.

Oligosaccharide thin layer chromatography can be used to detect abnormal excretion of oligosaccharides that occur in the oligosaccharidoses.

Confirmation of these results requires enzyme analysis of plasma, blood spots, leucocytes or skin fibroblasts, as appropriate, and for many disorders (eg Tay-Sachs Disease) this is the first line test.

Urinary GAG: Screening Test



The method exploits the specific interaction between the sulphated polymers of GAG and the tetravalent cationic dye Alcian blue.

GAGs can be detected semi-quantitatively by paper spot test or quantitatively by spectrophotometric monitoring of the GAG/dye complex formation using other structurally similar dyes to Alcian blue e.g. toluidine blue or 1,9-dimethylmethylene blue

Pitfalls

- Dilute urines produce unreliable results
- An Increased GAG excretion maybe non-specific
- Some (older) patients may have apparently normal GAG excretion

Further tests should be pursued if

- The GAG excretion is elevated for age
- The patient has strong clinical features of a mucopolysaccharidosis but GAG excretion is normal

Urinary GAG: 2Dimensional Electrophoresis



In this method urinary GAGs are precipitated by Alcian Blue, the extracted GAGs are then spotted on cellulose acetate sheets. 2D electrophoresis is performed sequentially using pyridine/acetic acid as the first solvent and 0.1M barium acetate as the second solvent. The cellulose acetate sheets are stained with Alcian Blue, washed and dried, and the patterns reviewed by experienced staff.

Advantages

- Identification of GAG species
- Semi-quantitation of individual GAG species for treatment monitoring

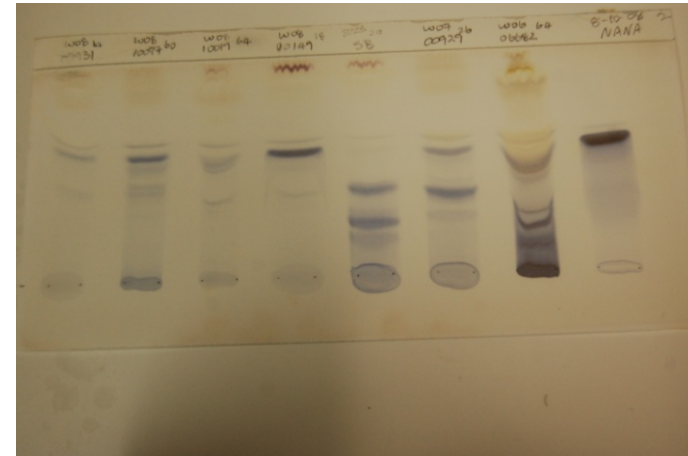
Pitfalls

- Many laboratories perform 1D electrophoresis as a first line test. Keratan sulphate (present in MPS IV, Morquio's Syndrome) can be difficult to visualise with this test so one needs to do 2D electrophoresis to exclude this disorder
- It requires experienced staff to review the patterns

Urinary Oligosaccharides & Sialic Acid



Oligosaccharides



Sialic acid

Both oligosaccharides and sialic acid (free and bound) can be detected on thin layer chromatography using the same type of silica gels but with slightly different solvents and different stains. For practical reasons they are usually run concurrently.

Advantage:

- This test can give a relatively clear indication for certain LSD such as GM1 gangliosidosis, alpha and beta mannosidosis, neuraminidase deficiency and sialic acid storage disease

Pitfalls

- Dilute urine may produce unreliable results
- There may be artefacts due to diet, drug, age-related pattern, jaundice
- It requires experienced staff to review the patterns

Specific Enzyme Assays

Sample types:

- Leucocytes isolated from 5-10ml EDTA blood can be used to screen for a number of LSD.
- Plasma – for certain LSD
- Fibroblasts
- Tissues
- Bloodspots – eg Pompe Disease and Fabrys Disease

Purposes:

1. The follow up of an abnormal result from glycosaminoglycan electrophoresis or oligosaccharide chromatography
2. Where there is a strong suspicion of a specific LSD based on clinical and radiological features, this often involves discussion between the laboratory and the physician
3. For the diagnosis of LSDs with no reliable urine test e.g. Sphingolipidoses
4. A known family history

Pitfalls

- The assays are time consuming and only performed by specific reference laboratories
- Pseudodeficiency states – Some individuals have very low (but detectable) levels of activity but are clinically asymptomatic. This often occurs with artificial substrates but not with the natural substrate.

Mucopolysaccharidoses (MPS)

MPS describes a subgroup of LSD characterised by accumulation of mucopolysaccharides, which are glycosaminoglycans attached to a link protein with a hyaluronic acid core. Deficiencies of single lysosomal enzymes which degrade these macromolecules lead to accumulation of heparan sulphate (HS), dermatan sulphate (DS) and keratan sulphate (KS). These are by-products of an incomplete degradation process and are excreted in the urine. According to the type and amount of these present in the urine, MPS can be sub classified into the following:

MPS classes	Type of GAGs accumulated
MPS I (Hurler, Scheie, Hurler/Scheie)	HS, DS
MPS II (Hunter)	HS, DS
MPS III (Sanfilippo)	HS
MPS IV (Morquio)	KS
MPS VI (Maroteaux-Lamy)	DS
MPS VII (Sly)	DS, HS
Multiple sulphatase deficiency	HS, DS, KS

MPS

There are 3 main presenting symptoms:	Type of MPS
Dysmorphic features	Hurler (MPS I) Hunter (MPS II) Maroteau-Lamy (MPS VI)
Learning difficulties; behavioural disturbances; dementia	Sanfilippo (MPS III)
Severe bony dysplasia	Morquio (MPS IV)

MPS have a wide spectrum of clinical severity. Hurler disease (MPS I) displays the full clinical picture of MPS and is the most severe form. Patients manifest within the first year with delayed psychomotor development and frequent ENT infections, very rapidly progressing to multi-organ involvement with hepatomegaly, bone deformities, coarse facial features, macrocephaly and corneal clouding. The finding of the bone abnormality dysostosis multiplex on radiology is characteristic of MPS I. The disease is fatal within the first two decades of life. The milder end of the spectrum is Scheie disease which is the adult variant of MPS I. Mild skeletal changes, stiff joints, corneal clouding, cardiac problems are present but mental retardation is not reported.

MPS III accounts for 80% of all MPS cases and is characterised by behavioural changes during school age. They usually have profound sleep disturbance, become hyperactive and aggressive, they lose acquired skills and exhibit developmental regression.

MPS

Diagnosis

Radiological and clinical examinations dominate the investigation process. Urinary GAG is increased in most cases but false negative results may be seen as explained previously. In light of a strong clinical suspicion further biochemical testing must be sought. Electrophoresis is performed to detect the presence of GAG species, the pattern of which will determine the specific lysosomal enzyme assays required. Finding of deficient enzyme activity is usually sufficient to confirm a diagnosis.

Treatment

This is mainly symptomatic and there is currently no cure. However enzyme replacement therapy (ERT) is available for MPS I, II, VI. There is good evidence that ERT could delay the non-neurological manifestations of the disease and improvement of quality of life. Bone marrow transplantation (BMT) has also been used with or without ERT in MPS I and VI patients who are asymptomatic and have the genotype associated with the very severe form of the disease. A clinical trial of ERT is currently underway for MPS IV.

Prenatal diagnosis is possible by measuring enzyme activity in chorionic villus biopsy or cultured amniotic fluid cells.

Oligosaccharidoses

The oligosaccharidoses have many clinical features similar to the MPS but are comparatively less common and do not have increased urinary GAG excretion. In this group of LSD, degradation of oligosaccharide chains in glycoproteins and glycolipids are impaired, resulting in the accumulation of oligosaccharides. There are four main causes:

1. Single enzymes defects in the degradation of oligosaccharides
(*E.g. Mannosidosis and Fucosidosis*)
2. The inability to synthesise the mannose-6-phosphate recognition marker so that newly synthesised enzymes will not be packaged into the lysosomes. Increased extracellular activities of some lysosomal enzymes can be detected.
(*E.g. Mucopolipidosis II and III*)
3. A defect in the lysosomal membrane protein sialin which transports sialic acid out of the lysosomes.
(*E.g. Salla disease, infantile free sialic acid storage disease*)
4. An absence of the protective protein Cathepsin A (PPCA) leading to an instability of the enzyme complex between neuraminidase and beta-galactosidase, which is subjected to rapid proteolytic degradation

Oligosaccharidoses

Clinical Presentations

Psychomotor retardation is the predominant sign in oligosaccharidoses, often associated with progressive neurological symptoms and seizures. Skeletal deformities and coarse facies vary from mild to severe. Hepatomegaly, deafness and corneal clouding may be absent, however some disorders show an unusual feature known as a cherry red spot on the retina. Early manifestation is more frequent than in the MPS. Some disorders present at birth or in the first year (hydrops foetalis, cardiomegaly) and are often fatal within a few years (or earlier). Severity may vary depending on the individual mutation.

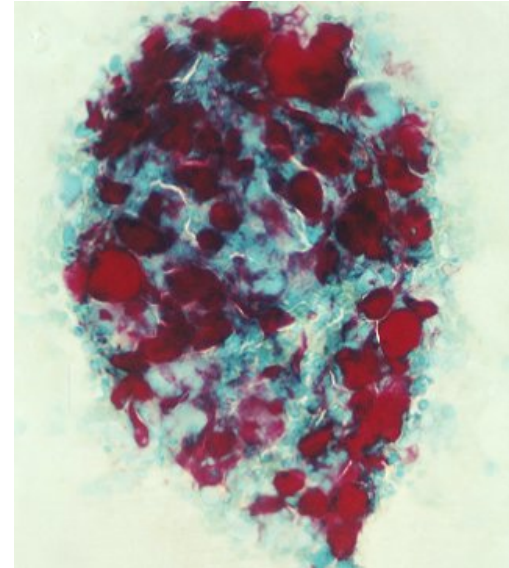
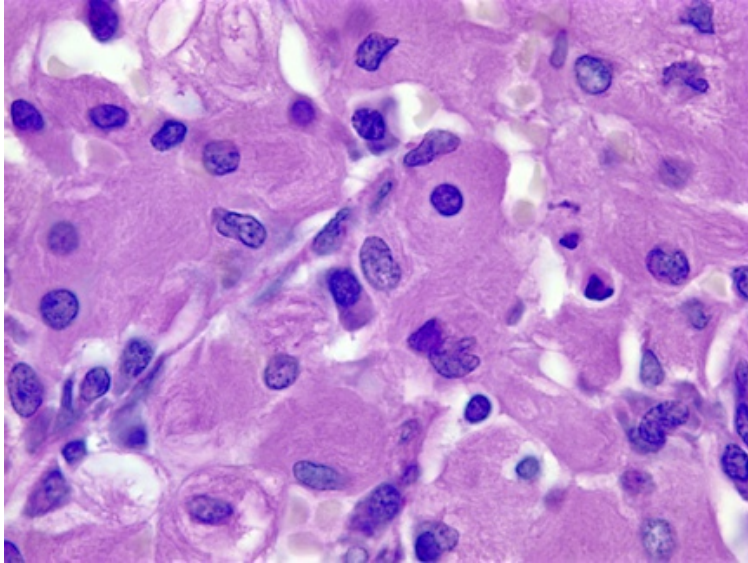
Diagnosis

Urinary oligosaccharides analysis by TLC is a useful screening test but this should be performed alongside urinary GAG electrophoresis in order to differentiate the oligosaccharidoses from the MPS. TLC allows identification of oligosaccharides and will also detect presence of sialic acid. The TLC pattern may indicate the appropriate enzyme tests to confirm a diagnosis.

Treatment

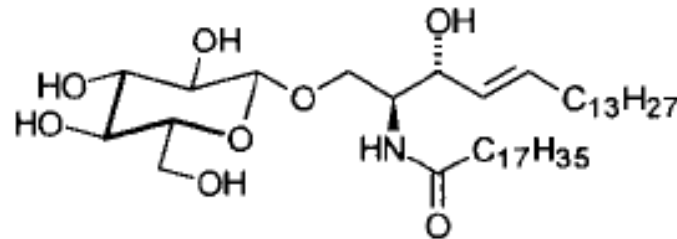
Currently there is no cure. Symptomatic relief and palliative care only.

Gaucher Disease



First described in 1882 by French physician Phillippe Gaucher. This is a lipid storage disease caused by a deficiency of the enzyme beta-glucocerebrosidase, resulting in accumulation of glucocerebroside in macrophage-monocytes system, liver and spleen. Gaucher cells are enlarged cells with a striated or 'wrinkled tissue paper' appearance and are seen in bone marrow aspirate and many other tissues. The enzyme hydrolyses the terminal glucose residue from these compounds. As a result glucocerebroside accumulates in the lysosome.

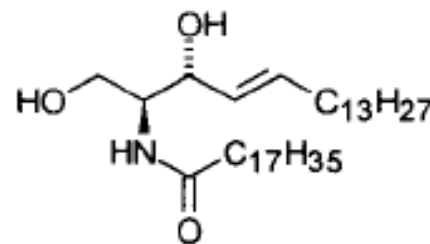
Gaucher : Enzyme Defect



Beta-Glucocerebroside
(Beta-Glucosylceramide)



Beta-Glucocerebrosidase
(*Beta-Glucosylceramidase*)
+
SAP-C (activator protein)



Ceramide

+ Glucose

Gaucher Disease

Gaucher disease is the commonest lysosomal storage disease. There are three clinical phenotypes:

Type I

The non-neuropathic form of Gaucher disease has a high prevalence among the Ashkenazi Jewish population. Patients may present in childhood with hepatosplenomegaly, pancytopenia, skeletal disease, whereas some may not be diagnosed until adulthood through the incidental finding of thrombocytopenia or splenomegaly. As the disease progresses, there is an increased incidence of cancer including lymphoma, myeloma and bone tumours. Some affected individuals may remain asymptomatic throughout life.

Type II

Acute neuropathic form with rapidly progressive neurovisceral storage disease and death usually occurring during infancy or in the first two years of life.

Type III

Chronic, slow progressive neuronopathic form. Death can occur in childhood or early adulthood. This form of Gaucher disease is more common in Norrbottnian region of Sweden, caused by deficiency of saposin (sap-C) which is an activator for the hydrolysis of glucocerebroside.

Neonatal form

Babies with this show colloidion skin or ichthyosis due to abnormal skin epidermal structures and hydrops. Perinatal or in utero death may occur.

Gaucher Disease

Diagnosis

The demonstration of increased plasma tartrate-resistant acid phosphatase and chitotriosidase (a marker of macrophage stimulation) and deficient glucocerebrosidase activity in lymphocytes/leukocytes, In sap-C deficiency (type III) the activator defect, the same findings are seen except that glucocerebrosidase activity is normal. Molecular analysis of the sap-C gene is available.

Molecular analysis

N370S is the commonest mutation on the glucocerebrosidase gene among the Ashkenazi Jewish population and is also frequent in Caucasian populations associated with the non-neuropathic phenotype. L444P is the second commonest mutation which is associated with type II and III.

Treatment

Bone marrow transplantation and ERT can be used for the non-neuronopathic type I and III. These are effective in slowing disease progression, especially bone and lung manifestations and also ameliorate the anaemia, thrombocytopenia, organomegaly, bone pain and crises. Substrate reduction therapy (SRT) is also available to patients with the milder form of type I. This works by slowing the synthesis of an accumulating by-product, which can be cleared by the residual enzyme activity remaining.

The patients' plasma chitotriosidase activities are monitored because it reflects the disease progression.

Tay Sachs Disease

Tay Sachs is caused by a deficiency of the alpha-subunit of beta-hexosaminidase A (HEX A), resulting in defective catabolism of GM2 ganglioside. The central nervous system is most affected by the accumulation of this ganglioside, leading to loss of CNS and motor function primarily due to neuronal damage. There are three forms:

Infantile form

The patients present at around 4-6 months with motor weakness and hypotonia. They produce a characterised startled response to sound with extension of the arms (hyperacusis). Visual impairment progresses to blindness and the finding of a cherry red spot in the retina is not uncommon. Macrocephaly, seizures, spasticity, swallowing difficulty and dementia soon follow. The infantile form is usually fatal by age 2-4 years.

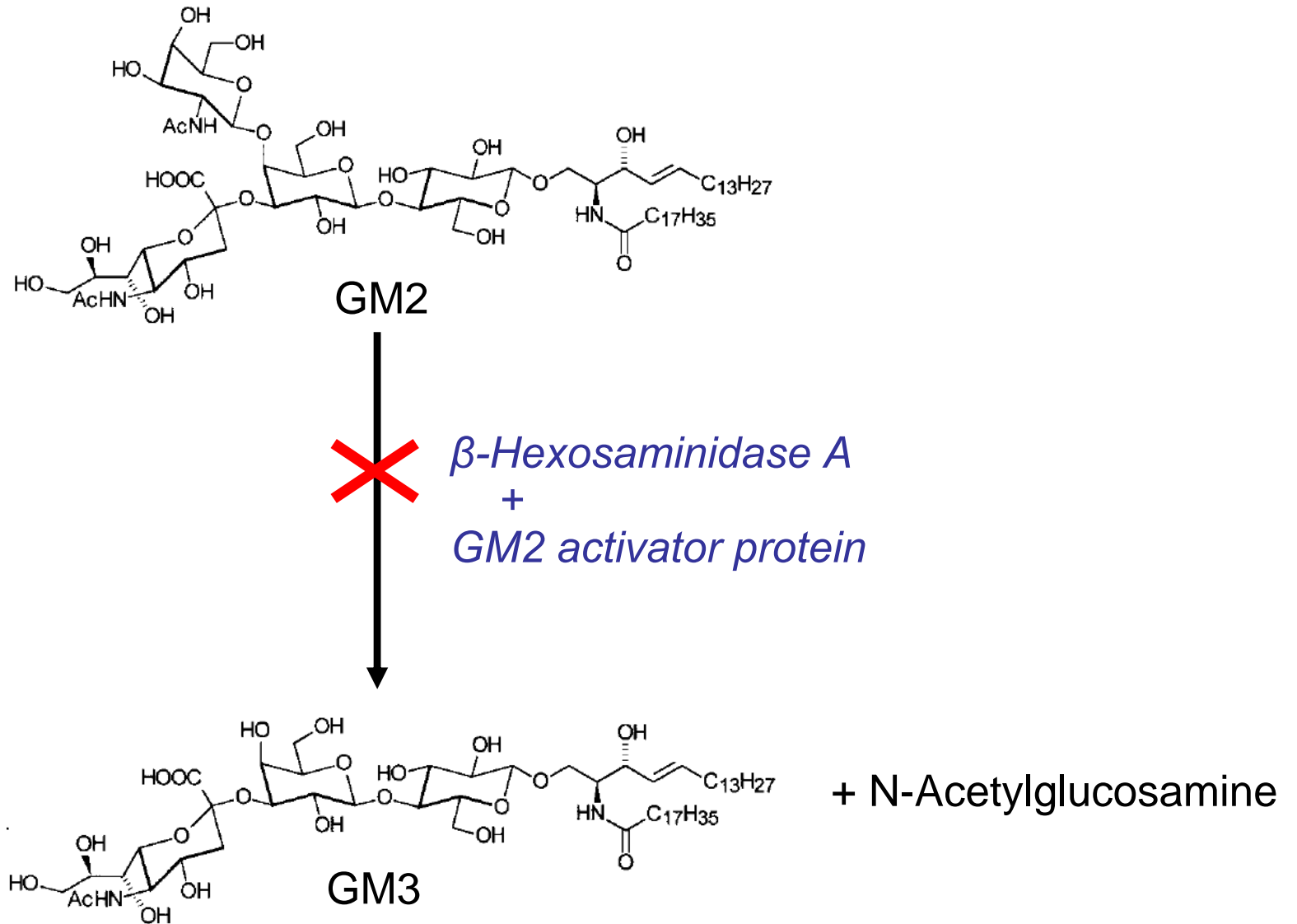
Late infantile and juvenile forms

Presenting symptoms are related to motor dysfunction; a cherry red spot and blindness may present. These are relatively aggressive forms and usually lead to death by 2-4 years of onset in the late infantile form, and in the juvenile form patients develop into a vegetative state and die in 10-14 years.

Adult or chronic form

A very rare form with unsteady gait and progressive neurological deterioration, some can survive into their 60-80's. A cherry red spot may not present.

Tay Sachs : Enzyme defect



Tay Sachs Disease

The incidence of Tay Sachs disease is particularly high among people of Eastern European and Ashkenazi Jewish descent. Among them the disease affects 1 in 3600 live births, and the carrier frequency is about 1 in 30.

Treatment

There is currently no cure for this disease, only symptomatic relief e.g. anticonvulsants, nutritional support, control of infections.

Diagnosis

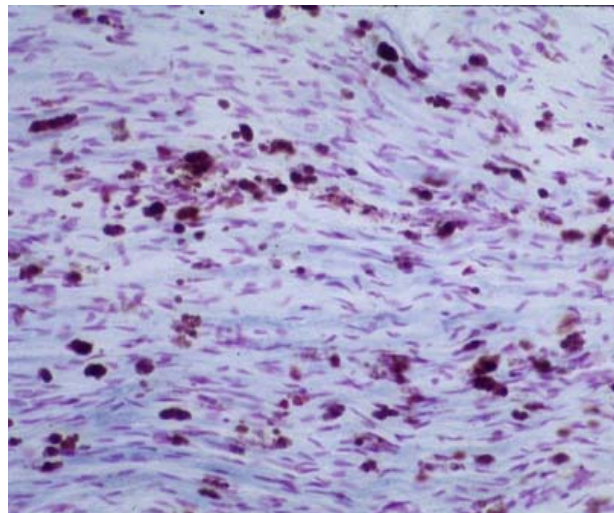
Measurement of beta-hexosaminidase A in plasma is used to diagnose this disorder in affected individuals. This may also be used to detect carriers in high risk populations. This enzyme assay can be performed on leucocytes and some laboratories offer testing on dried blood spots

Any abnormal results can be followed by DNA analysis to detect the disease-causing mutation or to exclude the presence of a pseudodeficiency allele.

Metachromatic Leucodystrophy

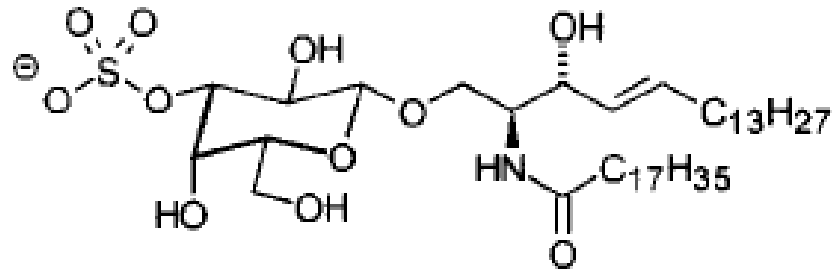
Metachromatic leucodystrophy (MLD) is caused by an inability to degrade sulphated glycolipids (especially the galactosyl-3-sulphate ceramides) due to a deficiency of cerebroside sulphatase (arylsulphatase A), or to a lack of an activator protein (sap-B) that is required for sulphatide degradation. Sulphatide compounds accumulate in neural tissue, destroying oligodendrocytes and Schwann cells and resulting in demyelination. It also accumulates in non-neural tissues, including the kidneys and gall bladder.

MRI of the brain shows bilateral and diffuse white matter changes. Signs of white matter involvement (spasticity, brisk tendon reflexes, extensor plantar responses) are prominent. Histological findings include presence of metachromatic granules in tissues, abnormal central and peripheral myelination. The accumulation of sulphatides causes extensive damage and is attributable to loss of both cognitive and motor functions.



Sulphatide stained with acid cresyl violet to form brown metachromasia in peripheral nerve

MLD : Enzyme defect



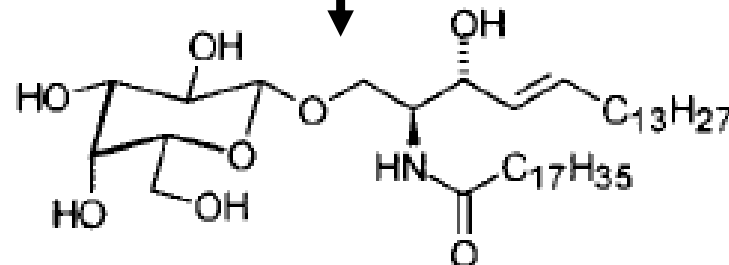
Sulphatide



Arylsulphatase A

+

SAP-B (activator protein)



Galactosylceramide

+ Sulphate

Metachromatic Leucodystrophy

There are four types of MLD that occur with varying ages of onset and courses. All forms of the disease involve a progressive deterioration of motor and neurocognitive function.

Late Infantile form (age of onset 1-2y)

This is the most common and most rapidly progressive form of MLD affecting both motor and cognitive function. Patients show gait disturbances, difficulty in walking, loss of motor developmental milestones, optic atrophy, and diminished deep tendon reflexes. This group of patients rarely survives beyond 5 years after onset.

Early juvenile (4-6y) and late juvenile forms (6-16y)

These show a less rapid disease progression. Behavioural and cognitive disturbances precedes motor dysfunction, especially in the late juvenile form. In some seizures may be the only presenting symptom. In the very rare cases they may present acutely with cholecystitis or pancreatitis secondary to gall bladder involvement. Death occurs before 20 years of age in most cases.

Adult form (>16y)

There are two distinct groups: one with predominantly motor function disturbances, pyramidal and cerebellar signs, dystonia and peripheral neuropathy. The other group is associated with behavioural and psychiatric problems, and sometimes misdiagnosed as psychiatric disorders.

Metachromatic Leucodystrophy

Diagnosis

Measurement of arylsulphatase A (ASA) activity in leukocytes or cultured fibroblasts is the initial diagnostic test using an artificial substrate. However 1-2% of the European population will show about 5-10% of normal ASA activity with no clinical symptoms. This is due to a defect in the polyadenylation signal for the gene leading to a reduction in the major mRNA species. This pseudodeficiency state can be excluded by detection of this known mutation.

Patients with MLD due to sap-B deficiency (the activator defect) will not show a deficiency of ASA. Further biochemical tests i.e. urinary glycolipid analysis and labelled sulphatides loading studies in fibroblasts are useful or alternatively mutation screening of the sap-B gene may be performed.

Treatment

There is evidence that BMT may slow disease progression and improve cognitive functions in some patients with the adult and juvenile forms of MLD. BMT in the pre-symptomatic affected sibling with the late infantile form have showed improvements in terms of survival and CNS involvement compared to the untransplanted sibling. BMT has no effect on the peripheral neuropathy.

LSD - Summary

Investigations of LSD

- These almost always begins with clinical suspicion, supported by clinical and radiological examinations
- This is followed by biochemical or histochemical detection and identification of the specific lysosomal storage material where this is possible.
- Confirmation is by assay of specific lysosomal enzymes to identify the point defect
- Molecular analysis available to some LSD with known common mutations e.g. Krabbe and Niemann-Pick C, and maybe useful in treatment decisions

Take home message

- It is difficult to distinguish clinically between the different types of LSD, common symptoms are useful to be aware of include coarse facies, corneal clouding, joint stiffness, hepatosplenomegaly, skeletal abnormalities, dementia, mental retardation and seizures
- Normal biochemical metabolite analyses do not exclude the diagnosis so it is important to pursue further testing in presence of strong clinical features of LSD
- A discussion with the reference laboratory is highly advisable

Questions

What is the major storage metabolites in the following LSDs?

1. Gauchers Disease
2. Hurler Syndrome
3. Mannosidosis
4. Tay-Sachs Disease
5. Morquio Syndrome
6. Metachromatic Leucodystrophy
7. Salla Disease

Answers

TYPE OF LSD

- Gauchers Disease
- Hurler Syndrome
- Alpha-Mannosidosis
- Tay-Sachs Disease
- Morquio Syndrome
- Metachromatic Leucodystrophy
- Salla Disease

STORAGE MATERIAL

- Beta-Glucocerebrosidase
- Glycosaminoglycans (DS & HS)
- Oligosaccharides
- GM2 Ganglioside
- Glycosaminoglycans (KS)
- Cerebroside Sulphate
- Sialic Acid